CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125553Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Clinical Pharmacology Review Amendment

BLA	125553
Submission Date:	May 8, 2014
Brand Name:	Zarxio
Proper Name:	To be determined
Formulation:	Intravenous and Subcutaneous solution
OCP Reviewer:	Sarah J. Schrieber, PharmD
OCP Team Leader:	NAM Atiqur Rahman, PhD
Pharmacometrics Reviewer:	Anshu Marathe, PhD
Pharmacometrics Team Leader:	Vikram Sinha, PhD
OCP Division:	DCP-V
OND Division:	OHOP\Division of Hematology Products (DHP)
Sponsor:	Sandoz
Submission Type; Code:	BLA 351(k)

This review serves as an amendment to the original clinical pharmacology review for BLA 125553 submitted to DARRTS on January 27, 2015.

For studies EP06-109, EP06-101, EP06-103, and EP06-105, the statistical analyses results using the 90% CI, 80-125% limits for the single dose absolute neutrophil counts (ANC) and multiple dose $CD34^+$ cell counts AUEC and Emax parameters are presented in the table below. The 90% CI for AUEC and ANC_{max} for ANC after single dose were within the limits of 80-125%. The 90% CI for AUEC and $CD34_{max}$ for $CD34^+$ cell counts after multiple doses (7 daily doses) were within the limits of 80-125%.

Study	Dose (mcg/kg)	ANC Geometric Mean Ratio (90% CI)	$CD34^+$ Geometric Mean Ratio (90% CI)
US-licensed Neupogen study			
EP06-109	10	$AUEC_{0\text{-}120h}$: 104 (101, 106) ANC_{max}: 101 (97, 104)	Multiple dose $CD34^+$ not evaluated.
EU-approved Neupogen studies			
EP06-105	1	$AUEC_{last}$: 102 (98, 107) ANC_{max}: 100 (95, 106)	Multiple dose $CD34^+$ not evaluated.
EP06-103	2.5	$AUEC_{0\text{-}24h}$: 105 (100, 109) ANC_{max}: 104 (98, 110)	$AUEC_{0\text{-}216h}$: 104 (98, 112) $CD34_{max}$: 106 (98, 114)
	5	$AUEC_{0\text{-}24h}$: 101 (96, 105) ANC_{max}: 100 (95, 104)	$AUEC_{0\text{-}216h}$: 99 (89, 110) $CD34_{max}$: 99 (86, 114)
EP06-101	10	Single dose ANC not reported	$AUEC_{0\text{-}216h}$: 110 (102, 118) $CD34_{max}$: 113 (103, 123)

Ratio (%): EP2006/US- or EU-Neupogen

The data presented in this amendment do not alter the recommendation of the original clinical pharmacology review of BLA 125553, which was submitted to DARRTS on January 27, 2015.

Signatures:

Reviewer: Sarah J. Schrieber, PharmD
Division of Clinical Pharmacology 5

Division Director: Nam Atiqur Rahman, PhD
Division of Clinical Pharmacology 5

Reviewer: Anshu Marathe, PhD
Division of Pharmacometrics

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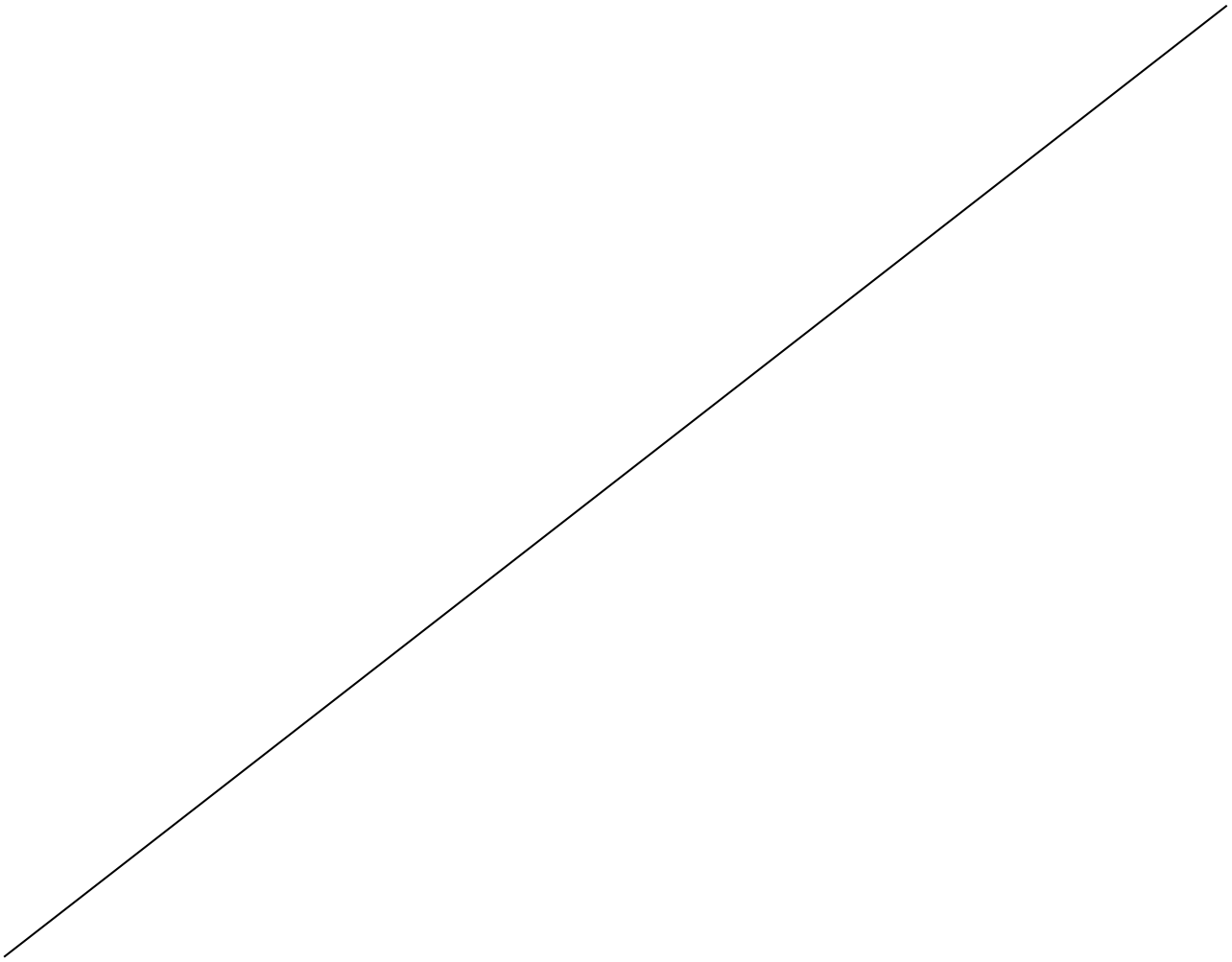
DCP-5: Reviewers - SJ Schrieber (CP), A Marathe, (PM)
CP TL – A Rahman; PM TL – V Sinha
DDD - B Booth DD - A Rahman



Attachment 1. The sponsor's November 5, 2014 response to FDA's October 31, 2014 information request.

1 Request for information 1

For studies EP06-109, EP06-101, EP06-103, and EP06-105, complete statistical analyses using the 90% CI, 80-125% limits for the single dose ANC and multiple dose $CD34^+$ PD AUEC and E_{max} parameters.

Sandoz response

Table 1-1 provides the results for the following pharmacodynamic (PD) parameters: $AUEC_{0\text{-last}}$ and E_{max}, for the absolute neutrophil count (ANC) and for the $CD34^+$ cell profiles. The ANC parameters are reported following a single dose administration of investigational medicinal product (IMP), and the $CD34^+$ cell parameters are reported following a multiple dose administration of IMP, except for study EP06-109 which was a single-dose study only. A point estimate and the corresponding 90% CI for the difference between EP2006 and Neupogen were calculated and were anti-logged to obtain the point estimate and the 90% CI for the ratio of the geometric means on the untransformed scale. The PD results for the comparisons show that the point estimate and all 90% CI are within the standard equivalence criteria of 80% to 125%.

Table 1-1 Summary of pharmacodynamic results

Study	Dose[a] [mcg/kg]	PD Parameter	Geometric Ratio[b]	Lower 90% CI	Upper 90% CI
EP06-101	10	Single Dose: AUEC - ANC	99.65	92.96	106.82
	10	Single Dose: E_{max} - ANC	102.26	95.67	109.30
	10	Multiple Dose: AUEC - $CD34^+$	109.77	102.05	118.07
	10	Multiple Dose: E_{max} - $CD34^+$	112.54	103.33	122.58
EP06-103	2.5	Single Dose: AUEC - ANC	104.47	99.85	109.32
	5	Single Dose: AUEC - ANC	100.68	96.18	105.40
	2.5	Single Dose: E_{max} - ANC	103.70	98.08	109.64
	5	Single Dose: E_{max} - ANC	99.83	95.47	104.39
	2.5	Multiple Dose: AUEC - $CD34^+$	104.49	97.82	111.62
	5	Multiple Dose: AUEC - $CD34^+$	98.98	88.75	110.39
	2.5	Multiple Dose: E_{max} - $CD34^+$	105.71	98.19	113.81
	5	Multiple Dose: E_{max} - $CD34^+$	99.24	86.32	114.10
EP06-105	1	Single Dose: AUEC - ANC	102.29	98.02	106.75
	1	Single Dose: E_{max} - ANC	100.38	95.30	105.72
EP06-109	10	Single Dose: AUEC - ANC	103.54	101.45	105.67
	10	Single Dose: E_{max} - ANC	100.71	97.45	104.08
	10	Single Dose: AUEC - $CD34^+$	102.56	95.48	110.16
	10	Single Dose: E_{max} - $CD34^+$	104.65	93.72	116.86

[a] Study EP06-103 was the only study including more than 1 dose group.

[b] Geometric ratio is the ratio of means for EP2006/Neupogen.

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/s/

SARAH J SCHRIEBER
02/09/2015

ANSHU MARATHE
02/12/2015

VIKRAM P SINHA
02/12/2015

NAM ATIQUR RAHMAN
02/12/2015

Division Director Summary Review

Date	January 30, 2015
From	NAM Atiqur Rahman, Ph.D. Division Director, Division of Clinical Pharmacology V Office of Clinical Pharmacology
Subject	Division Director's Memo
BLA #	125553
Applicant's Name	Sandoz, Inc.
Proposed Indication(s)	All Indications for which US-Licensed Neupogen is currently licensed
Recommendation	Clinical Pharmacology data contribute to the totality of evidence and support the approval of the product for all indications

Sandoz Inc. submitted a Biologic License Application (BLA) for EP2006 under section 351(k) of the Public Health Service Act as a biosimilar product to the US-licensed product Neupogen which is marketed by Amgen Inc. The clinical pharmacology data contribute substantially to the determination that there are **"no clinically meaningful differences"** between EP2006 and the reference product in terms of safety, purity, and potency of the product. These data contribute to the totality of evidence needed to support the approval of EP2006 as a biosimilar product to US-licensed product Neupogen.

EP2006 was approved by the EMA in 2009 and is now marketed in over 60 countries worldwide, which has resulted in a clinical exposure of more than 7.5 million patient-days (Sandoz ODAC presentation, January 7, 2015).

Granulocyte Colony Stimulating Factor (G-CSF) is a non-glycosylated, single amino acid chain, 18.8 KDa protein. Its structure is simpler than the pegylated proteins and antibodies that are marketed as therapeutic proteins. Neutrophils, the most abundant granulocytes, are depleted in patients treated with myelosuppressive therapy. The depletion of neutrophils leads to various types of infections manifested by febrile neutropenia that require intravenous antibiotic usage and hospitalization. G-CSF is a useful treatment modality for these patients because it causes hematopoietic recovery and immune response. G-CSF mediates its action by binding to the G-

CSF receptors that are present on the precursor cells in the bone marrow and initiates proliferation and differentiation of the precursor cells into mature granulocytes. The intensity and duration of severe neutropenia (neutrophil count ≤ 500/mL) correlate with the incidence and severity of infection. Duration of severe neutropenia (DSN) is an important clinical endpoint that determines efficacy of G-CSF products. Absolute neutrophil count (ANC) in blood is a measure that relates with DSN and it is considered an acceptable pharmacodynamics (PD) marker for neutropenia-related indications.

Binding of G-CSF to its receptor also causes mobilization of hematopoietic progenitor cells into the peripheral blood, which are collected by leukapheresis and transplanted into patients. The success of the mobilization of hematopoietic progenitor cells is demonstrated by the total number of Colony Forming Unit-Granulocyte, Monocyte (CFU-GM) and/or $CD34^+$ cells collected by leukapheresis for engraftment. Therefore, $CD34^+$ cell count is a PD marker for mobilization indication of G-CSF.

Sandoz developed a PK- (pharmacokinetics) and PD-based clinical program to assess the similarity of EP2006 and support the demonstration of no clinically meaningful difference in safety, purity, and potency of EP2006 compared with the US-licensed product Neupogen. The sponsor received advice from the Agency during the development of EP2006 for the US market and the program reflects evolving FDA scientific advice, which is currently reflected in the draft guidance for industry titled, "Clinical Pharmacology Data to Support Demonstration of Biosimilarity to a Reference Product" published in May 2014. Similar advice is currently being given to sponsors developing proposed biosimilar G-CSF products for marketing in the United States.

Sandoz submitted four clinical studies that evaluated single and multiple subcutaneous (SC) doses between 1 and 10 mcg/kg in healthy subjects. The objectives of these studies were to establish the PK and PD similarity of EP2006 with US-licensed Neupogen. Among these, three studies used EU-approved Neupogen. A 3-way comparison of the analytical similarity of critical quality attributes (analytical bridge) of EP2006, US-licensed Neupogen, and EU-approved Neupogen justified the relevance of the clinical PK and PD data generated using EU-approved Neupogen. Overall, the clinical studies demonstrated PK and PD similarity between EP2006 and US-licensed Neupogen based on the 90% confidence interval (CI) for the geometric mean ratio (GMR) of area under the plasma concentration and time curve (AUC) and maximum plasma concentration (C_{max}) within the pre-specified limits of 80 to 125% and the 95% CI for the GMR of area under the effect curve (AUEC) and maximum concentration of ANC and $CD34^+$ within the pre-specified 80 to 125% limits. The Advisory Committee (Oncology Drug Advisory Committee, ODAC) held on January 7, 2015 agreed with the review team's conclusion that the PK and PD study results added to the totality of the evidence to support a demonstration of biosimilarity of

EP2006 and US-licensed Neupogen and recommended that EP2006 should receive licensure as a biosimilar product for all the indications for which US-licensed Neupogen is currently licensed.

The clinical pharmacology studies of EP2006 consisting of PK similarity at single SC doses ranging from 1 to 10 mcg/kg and PD similarity at multiple SC doses ranging from 2.5 to 10 mcg/kg in healthy subjects using ANC and $CD34^+$ as PD markers is sensitive and relevant and addresses the residual uncertainties remaining after the analytical similarity assessment. The single dose and multiple dose PK and PD data were critical elements in the EP2006 program to support both the neutropenia and mobilization indications. The comparative clinical data in breast cancer patients further supported the conclusions drawn from the PK and PD studies in healthy volunteers. The PK and PD development program is consistent with the scientific expectations as articulated in the draft guidance for industry titled, "Clinical Pharmacology Data to Support Demonstration of Biosimilarity to a Reference Product." The marketing experience of EP2006 in over 60 countries and clinical experience of more than 7.5 million patient-days indicate no major safety issues. The Divisions of Clinical Pharmacology V and Pharmacometrics in the Office of Clinical Pharmacology (OCP) conclude that the clinical pharmacology data provides compelling evidence of no clinically meaningful difference in safety, purity, and potency of EP2006 and the US-licensed Neupogen and recommends that the product should be approved. Sandoz should provide a comprehensive summary of post marketing safety and immunogenicity data of EP2006 generated in countries where this product is marketed.

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/s/

NAM ATIQUR RAHMAN
02/05/2015

Clinical Pharmacology Review

BLA	125553
Submission Date:	May 8, 2014
Brand Name:	Zarxio
Proper Name:	To be determined
Formulation:	Intravenous and Subcutaneous solution
OCP Reviewer:	Sarah J. Schrieber, PharmD
OCP Team Leader:	NAM Atiqur Rahman, PhD
Pharmacometrics Reviewer:	Anshu Marathe, PhD
Pharmacometrics Team Leader:	Vikram Sinha, PhD
OCP Division:	DCP-V
OND Division:	OHOP\Division of Hematology Products (DHP)
Sponsor:	Sandoz
Submission Type; Code:	BLA 351(k)
Dosing regimen:	5 mcg/kg and 10 mcg/kg
Indications:	Same as those for US-licensed Neupogen: -Cancer patients receiving myelosuppressive chemotherapy -Patients with acute myeloid leukemia receiving induction or consolidation chemotherapy -Cancer patients receiving bone marrow transplant -Patients undergoing peripheral blood progenitor cell collection and therapy -Patients with severe chronic neutropenia

Table of Contents

EXECUTIVE SUMMARY

This Biologic License Application (BLA) for EP2006 (recombinant human granulocyte stimulating factor (G-CSF)) has been submitted under Section 351(k) of the Public Health Service Act (42 U.S.C. 262(k)). The applicant is seeking approval for EP2006 as a proposed biosimilar to US-licensed Neupogen licensed under BLA 103353 by Amgen Inc., and is seeking licensure for all the indications for which US-licensed Neupogen is currently approved. EP2006 drug product was developed as a liquid for injection, filled in a pre-filled syringe in the strengths of 300 mcg/0.5 mL and 480 mcg/0.8 mL.

The applicant submitted four pharmacokinetic (PK) and pharmacodynamic (PD) studies that evaluated subcutaneous (SC) doses of 1-10 mcg/kg in healthy subjects to evaluate the PK and PD similarity of EP2006 with US-licensed Neupogen. In addition to PK, these studies evaluated absolute neutrophil counts (ANC) and $CD34^+$ cell counts as relevant and sensitive PD markers for the similarity assessment. Among these, three studies utilized EU-approved Neupogen. As such, adequate data and information was needed to scientifically justify the relevance of these comparative data to an assessment of biosimilarity to the US-licensed reference product. The pairwise comparisons of EP2006, US-licensed Neupogen, and EU-approved Neupogen met the pre-specified criteria for analytical similarity and established a scientific bridge to justify the relevance of the PK/PD data generated using EU-approved Neupogen (refer to CMC review).

The 90% CI for AUC and C_{max} after a single dose were within the pre-defined limits of 80-125%. Similarly, the 95% CI for AUEC and ANC_{max} for ANC after a single dose were within the pre-defined limits of 80-125%. The 95% CI for AUEC and $CD34_{max}$ for $CD34^+$ cell counts after multiple doses were within the limits of 80-125%.

Overall, the PK and PD studies support a demonstration of PK and PD similarity between EP2006 and US-licensed Neupogen. The PK and PD studies results add to the totality of the evidence to support a demonstration of biosimilarity of EP2006 and US-licensed Neupogen.

1.1 RECOMMENDATIONS

The Office of Clinical Pharmacology has determined that the PK and PD results support a demonstration of no clinically meaningful differences between EP2006 and US-licensed Neupogen and recommends approval of EP2006.

Labeling Recommendations

Please refer to Section 2 - Detailed Labeling Recommendations.

Phase IV Requirements

None.

Signatures:

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Team Leader: Nam Atiqur Rahman, PhD
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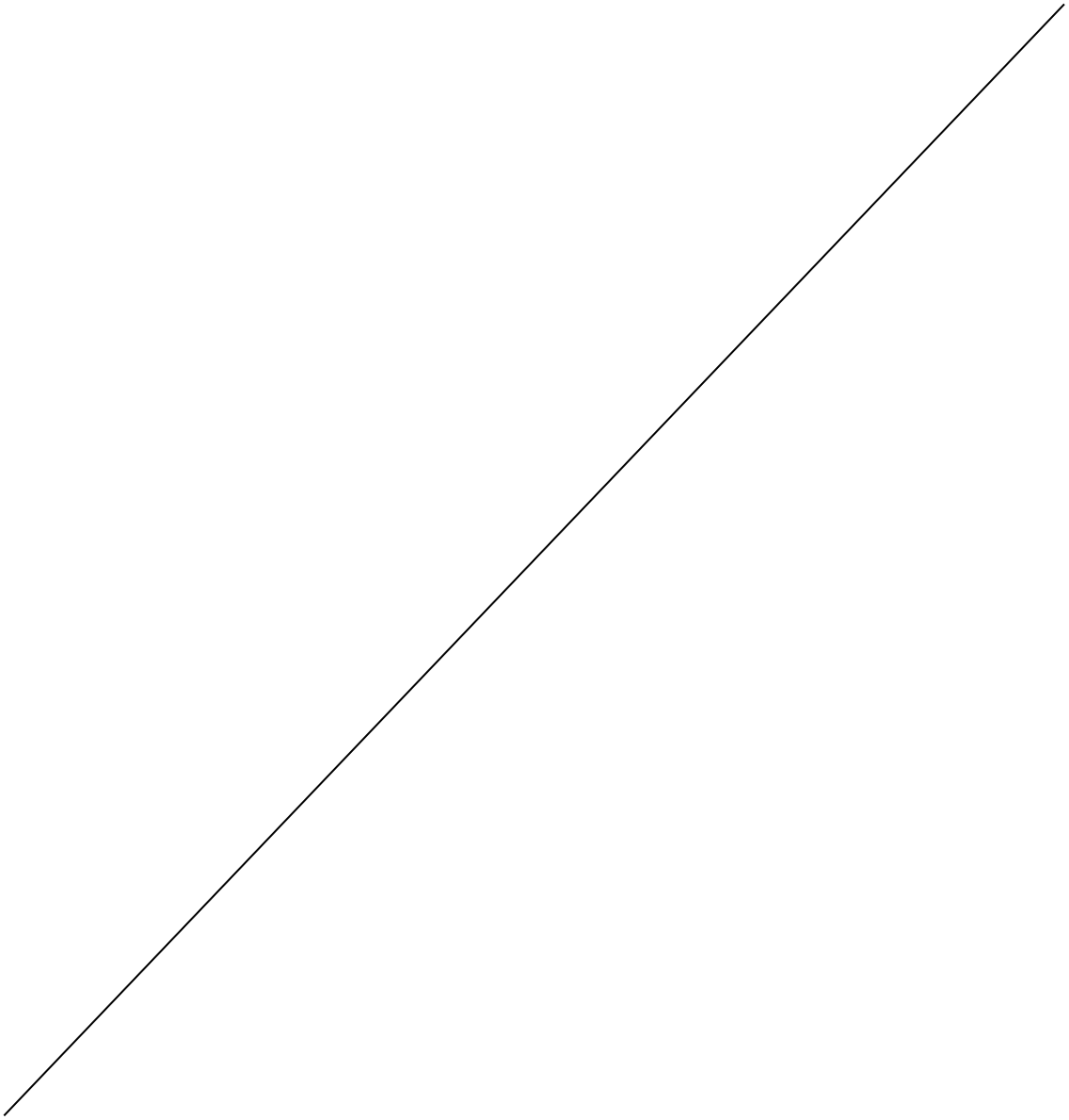
CP TL – A Rahman; PM TL – V Sinha
DDD - B Booth DD - A Rahman



1.2 SUMMARY OF CLINICAL PHARMACOLOGY FINDINGS

This Biologic License Application (BLA) for EP2006 (recombinant human granulocyte stimulating factor (G-CSF)) has been submitted under Section 351(k) of the Public Health Service Act (42 U.S.C. 262(k)). The applicant is seeking approval for EP2006 as a biosimilar product to the US-reference product Neupogen® licensed under BLA 103353 by Amgen Inc. for the same indications. EP2006 drug product was developed as a liquid, injection, filled in a pre-filled syringe in the strengths of 300 mcg/0.5 mL and 480 mcg/0.8 mL.

The applicant submitted four pharmacokinetic (PK) and pharmacodynamic (PD) studies that evaluated subcutaneous (SC) doses of 1-10 mcg/kg in healthy subjects to evaluate the PK and PD similarity of EP2006 with US-licensed Neupogen. In addition to PK, these studies evaluated absolute neutrophil counts (ANC) and CD34+ cell counts as relevant and sensitive PD markers for biosimilarity assessment. Among these, three studies utilized EU-approved Neupogen. As such, adequate data and information was needed to scientifically justify the relevance of these comparative data to an assessment of biosimilarity to the US-licensed reference product. The pairwise comparisons of EP2006, US-licensed Neupogen and EU-approved Neupogen met the pre-specified criteria for analytical similarity for the purposes of establishing a scientific bridge to justify the relevance of the data generated using EU-approved Neupogen (refer to CMC review).

The 90% CI for AUC and C_{max} after a single dose were within the pre-defined limits of 80-125%. The 95% CI for AUEC and ANC_{max} for ANC after single dose were within the pre-defined limits of 80-125%. The 95% CI for AUEC and $CD34_{max}$ for $CD34^{+}$ cell counts after multiple doses (7 daily doses) were within the limits of 80-125%.

Study	Dose (mcg/kg)	PK Geometric Mean Ratio (90% CI)	ANC Geometric Mean Ratio (95% CI)	$CD34^{+}$ Geometric Mean Ratio (95% CI)
US-licensed Neupogen study				
EP06-109	10	AUC_{last}: 88 (84, 91) C_{max}: 88 (84, 92)	$AUEC_{0\text{-}120h}$: 102 (97, 109) ANC_{max}: 100 (94, 105)	Multiple dose CD34+ not evaluated.
EU-approved Neupogen studies				
EP06-105	1	$AUC_{0\text{-}36h}$: 91 (86, 97) C_{max}: 89 (82, 96)	$AUEC_{last}$: 103 (100, 106) ANC_{max}: 100 (96, 103)	Multiple dose CD34+ not evaluated.
EP06-103	2.5	$AUC_{0\text{-}24h}$: 88 (81, 85) C_{max}: 87 (79*, 95)	$AUEC_{0\text{-}24h}$: 102 (99, 105) ANC_{max}: 104 (97, 111)	$AUEC_{0\text{-}216h}$: 105 (97, 113) $CD34_{max}$: 99 (84, 117)
	5	$AUC_{0\text{-}24h}$: 96 (90, 102) C_{max}: 96 (89, 104)	$AUEC_{0\text{-}24h}$: 101 (98, 103) ANC_{max}: 100 (95, 105)	$AUEC_{0\text{-}216h}$: 99 (87, 113) $CD34_{max}$: 99 (84, 117)
EP06-101	10	$AUC_{0\text{-}24h}$: 93 (89, 98) C_{max}: 89 (82, 96)	Single dose ANC not reported	$AUEC_{0\text{-}216h}$: 102 (95, 110) $CD34_{max}$: 99 (90, 110)

*The lower limit for Cmax at the 2.5 mcg/kg dose fell just outside the 80-125% range.
Ratio (%): EP2006/US- or EU-Neupogen

Overall, the PK and PD studies support a demonstration of PK and PD similarity between EP2006 and US-licensed Neupogen.

2 QUESTION BASED REVIEW

2.1 GENERAL ATTRIBUTES

2.1.1 What are the proposed dosage(s) and route(s) of administration?

The applicant proposes the same dosing regimen of 5 mcg/kg and 10 mcg/kg IV or SC daily, which is approved for US-licensed Neupogen.

2.2 GENERAL CLINICAL PHARMACOLOGY

2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

The PK and PD of EP2006 following SC administration have been characterized in studies that include either US-licensed Neupogen or EU-approved Neupogen as the comparator to support the Clinical Pharmacology Section of the BLA submission (Table 1). The PK and PD studies were considered critical in the assessment of no clinically meaningful difference in safety, purity, and potency between EP2006 and US-licensed Neupogen.

Table 1. Summary of relevant EP2006 clinical studies.

Study (Dates)	Design Features	Objectives	Dose/Route/Duration
Studies using US-licensed Neupogen (the reference product)			
EP06-109 (25-Feb-2011 to 22-Apr-2011)	Randomized, double-blind 2-way crossover in HS (N=26)	1. ANC ($AUEC_{0-120h}$ and ANC_{max}), PK (AUC_{0-last} and C_{max}) 2. $CD34^+$, safety	10 mcg/kg, SC single dose
EP06-302 (26-Dec-2011 to 17-Jun 2013)	Randomized double-blinded, active controlled study (N=204)	1. Safety, efficacy Included a Cycle 1 PK sub-study (n=54, 27/arm)	5 mcg/kg, SC multiple dose
Studies using EU-approved Neupogen			
EP06-103 (29-Aug-2006 to 5-Dec-2006)	Randomized, double-blind, 2-way crossover in HS, with two dose groups (N=28/dose)	1. ANC ($AUEC_{0-24h}$ and ANC_{max}) 2.PK (AUC_{0-24h} and C_{max}), $CD34^+$ ($AUEC_{0-216h}$ and $CD34_{max}$), safety	2.5 & 5 mcg/kg, SC single and multiple (7d) dose
EP06-105 (21-Apr-2008 to 26-May-2008)	Randomized, double blind, 2-way crossover in HS (N=23)	1. ANC ($AUEC_{0-24h}$ and ANC_{max}) 2. PK (AUC_{0-24h} and C_{max}), safety	1 mcg/kg, SC single dose
EP06-101 (25-Oct-2004 to 12-Apr-2005)	Randomized, double-blind, 2-way crossover in HS (N=32)	1. PK (AUC_{0-24h} and C_{max}) 2. $CD34^+$ ($AUEC_{0-216h}$ and $CD34_{max}$), ANC ($AUEC_{0-24h}$ and ANC_{max}), safety	10 mcg/kg, SC single and multiple (7d) dose
EP06-104 (19-Mar-2008 to 26-May 2008)	Randomized, double-blind, 3-way crossover in HS (N=28)	1. PK (AUC_{0-last} and C_{max}) 2. ANC ($AUEC_{0-last}$ and ANC_{max}), safety	2.5 mcg/kg , SC single dose -EP2006 (Glutamate and Acetate formulations) and EU-Neupogen (Acetate)

HS, healthy subjects; ANC; absolute neutrophil count; PK, pharmacokinetics; SC, subcutaneous

2.2.1.1 Efficacy & Safety Study

Study EP06-302 was a double-blind parallel group comparative clinical study in women with breast cancer who were eligible for neoadjuvant or adjuvant treatment were administered 6 cycles of TAC chemotherapy (Taxotere 75 mg/m^2, Adriamycin 50 mg/m^2 and Cytoxan 500 mg/m^2, all administered IV on Day 1 of each 21-day cycle). This study is considered supportive in the assessment of similarity between EP2006 and US-licensed Neupogen.

Starting 24 hours after the completion of chemotherapy, daily SC 5 mcg/kg US-licensed Neupogen or EP2006 was administered until the ANC recovered to 10 x 10^9/L after the nadir or up to a maximum of 14 days (which ever occurred first). The FDA review of efficacy in study EP06-302 focused on a comparison of duration of severe neutropenia (DSN) in Cycle 1 in patients treated with either EP2006 or US-licensed Neupogen, which was defined as the number of consecutive days in which the patient has an ANC <0.5 x 10^9/L following Cycle 1 of chemotherapy. The results from the FDA analysis are presented in Table 2. The analysis showed that EP2006 is equivalent to Neupogen in terms of efficacy as measured by the difference of DSN between US-Neupogen and EP2006 being less than 1 day for both the upper and lower bounds of the 90% CI. Refer to the clinical/statistics review for detailed information about this study.

Table 2. Cycle 1 DSN results (FDA analysis).

Primary Endpoint	**EP2006 (N=101)**	**US-Neupogen (N=103)**
Cycle 1 Mean DSN (SD)	1.17 days (1.11)	1.20 days (1.02)
DSN Difference for Neupogen minus EP2006 (90% CI)*	0.04 (-0.21, 0.28)*	

*ANCOVA with treatment, disease status and baseline ANC level

This study also included a PK substudy and the results are described in Section 2.2.5.1.

2.2.1.2 Clinical Pharmacology Studies

Data on the pharmacokinetics (PK) and pharmacodynamics (PD) of EP2006 is available from the studies listed in Table 1. The PK similarity studies enrolled healthy subjects for evaluation of PK and PD similarity of EP2006 to US-licensed Neupogen and EU-Neupogen. The comparative clinical study enrolled patients with breast cancer and assessed the safety and efficacy of EP2006 vs. US-licensed Neupogen.

Given that the applicant is seeking to use data from clinical studies comparing EP2006 to a non-US-licensed product to address, in part, the requirements under section 351(k)(2)(A) of the PHS Act, the applicant provided data and information to scientifically justify the relevance of these comparative data to an assessment of biosimilarity and established a scientific bridge to the US-licensed reference product; refer to CMC review for details.

Refer to Appendix 3.1 for background information and justifications regarding the use of the PK and PD endpoints, the use of healthy subjects in PK and PD studies, and the PK and PD study designs.

2.2.2 What is the basis for selecting the response endpoints (i.e., clinical or surrogate endpoints) or biomarkers (collectively called pharmacodynamics (PD) measures) and how are they measured in clinical pharmacology and clinical studies?
Pharmacokinetics (AUC and Cmax) was assessed following a single dose out to 24 to 48 hours post-dose, which captures at least 5 half-lives of G-CSF, and is acceptable.

For G-CSF, the PD measures are considered to be absolute neutrophil count (ANC) and $CD34^+$ cell count. These PD (ANC and $CD34^+$) measures reflect the mechanism of action of G-CSF for neutropenia and for peripheral blood progenitor cell mobilization, respectively. In study EP06-109, the single dose PD endpoints were ANC $AUEC_{0\text{-}120h}$ and ANC_{max}, which captured at least 80% of the ANC area under the effect curve (AUEC) profile, and is acceptable. In studies EP06-101 and EP06-103, the 7 day multiple dose PD endpoints were $CD34^+$ $AUEC_{0\text{-}216h}$ and $CD34_{max}$, which captured at least 80% of the $CD34^+$ AUEC profile, and is acceptable. The PD (ANC and $CD34^+$) assessments were adequate to determine similarity of EP2006 to the EU-approved Neupogen.

The primary efficacy endpoint in the comparative clinical study EP06-302 was duration of severe neutropenia (DSN), which was considered a clinically relevant endpoint for the approval of US-licensed Neupogen.

Based on data from the US-licensed Neupogen arm in Cycle 1 from the study EP06302, ANC AUEC is correlated with the primary endpoint of DSN, as shown in Figure 1. DSN decreases with increasing ANC AUEC. This correlation was quantified using a Poisson regression model. The model fit the data reasonably well as shown in Figure 2. The parameters from the Poisson regression model are summarized in Table 3.

Figure 1. Correlation between AUEC of ANC (day*10^9/L) and DSN (days) for US-licensed Neupogen in study EP06-302. AUEC represents the area under the ANC curve from day 1 to day 10. Patients were divided in 4 quartiles based on their ANC AUEC and their mean (SE) DSN were calculated and plotted for the 4 groups.

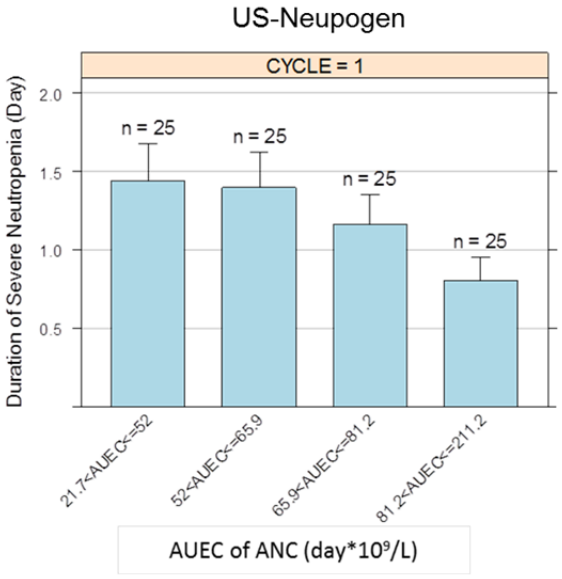


Figure 2. Observed and Predicted DSN versus AUEC of ANC. Diagnostic plot suggesting reasonable fit of the Poisson regression model to the observed data.

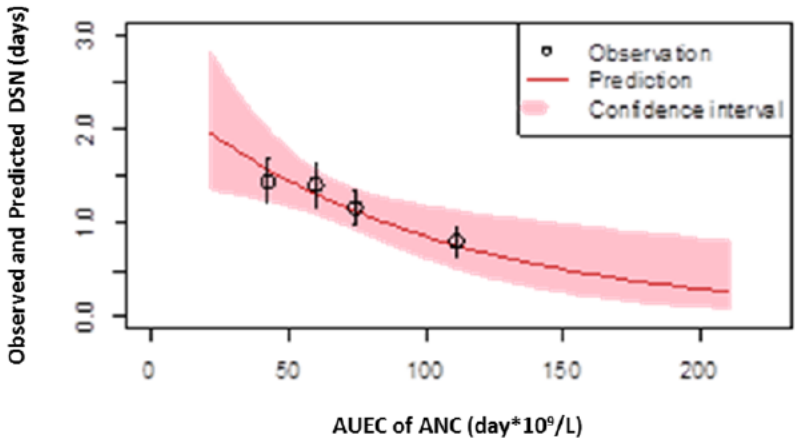


Table 3. Parameter Estimates of the Poisson Regression Model for DSN.

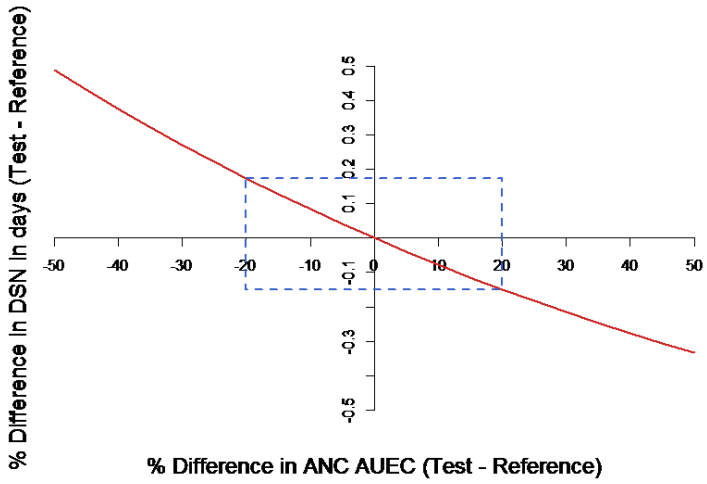
Parameter	Estimates	Std. Error	p-value
Intercept	0.897	0.262	0.0006
AUEC of ANC*	-0.0105	0.004	0.006

*With 10 unit increase (i.e., $10*10^9$ day/L increase) in AUEC of ANC, the mean DSN would decrease by a factor of exp(-0.0105*10)=0.9 (e.g., from 1 day to 0.9 day).

Furthermore, using the quantitative relationship developed, simulations were carried out to illustrate the sensitivity of ANC AUEC to detect potential clinically meaningful differences in effectiveness of products. For these simulations, the mean ANC AUEC was varied from -50% to 50%. Scenarios where the difference in the PD between two products (e.g., test and the reference product) varied across this range were simulated. The mean differences in DSN between the products were calculated using the Poisson model.

Simulation results, illustrated in Figure 3 show that a ± 20% difference in the ANC AUEC between the test and the reference would translate into a mean difference in DSN of less than ± 0.2 days between the products. A difference of ± 1 day between products is generally considered an acceptable margin for efficacy assessment of G-CSF products.

Figure 3. Plot to illustrate the sensitivity of ANC AUEC to detect clinically meaningful differences in effectiveness in terms of duration of severe neutropenia between products.



Refer to Appendix 3.1 for more details.

2.2.3 What are the PK and PD predefined similarity margins?

In all studies, the predefined similarity criteria for both AUC and C_{max} were that the 90% CI of the ratio should lie within 80-125% except for EP06-101 where a wider margin of 75-133% for C_{max} was pre-defined. The margin of 80-125% proposed by the applicant is acceptable.

For ANC, the predefined similarity criteria for both AUEC and ANC_{max} were that the 95% CI for the ratio of the geometric means should lie within 80-125% in studies EP06-109 and EP06-105. In study EP06-103, the applicant used tighter predefined criteria that 95% CI for the ratio should lie within 87.25-114.61% for the 2.5 mcg/kg dose and 86.50-115.61% for the 5 mcg/kg dose. The applicant also presented the 90% CI for the ratio of the geometric means of the exposure parameters for the test and reference products. The applicant's pre-defined criteria are acceptable as it is tighter and more conservative than the standard criteria of 90% CI of the ratio to lie within 80-125%.

For $CD34^{+}$, there were no predefined criteria for similarity. However, the applicant did report the 95% CI and 90% CI for the ratio of the exposure parameters (AUEC and $CD34_{max}$).

Refer to Appendix 3.1 for more details.

2.2.4 Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

Yes. G-CSF was measured to characterize the PK and was measured in serum by a validated enzyme-linked immunosorbant assay (ELISA).

2.2.5 Exposure Response

2.2.4.4 Are the dose(s) selected for PK and PD studies appropriate?

The dose(s) of EP2006 were selected based on the approved dose(s) of US-licensed Neupogen, where efficacy and safety has been previously confirmed (refer to the US-licensed Neupogen product labeling).

Regarding dose-exposure response for G-CSF PK and PD (ANC and $CD34^+$), increases in the SC dose of 1-10 mcg/kg elicits changes in both PK and PD in healthy subjects. Refer to Appendix 3.1 for more details.

2.2.6 Pharmacokinetic characteristics of the drug and its major metabolites

The PK of G-CSF was characterized by noncompartmental methods in each of the studies included in the application.

2.2.6.1 What are the single dose PK and PD (ANC) parameters and multiple dose PD ($CD34^+$) parameters in healthy volunteers?

Single dose PK and PD (ANC)

US-licensed Neupogen single dose PK results

Single dose PK was assessed in study EP06-109 where single 10 mcg/kg SC doses EP2006 and US-Neupogen were administered to healthy subjects. Table 4 summarizes the PK parameter values. The $AUC_{0\text{-last}}$ and C_{max} geometric mean ratios and 90% CI met the pre-specified 80-125% criteria (Table 5). Of note, $AUC_{0\text{-inf}}$ also met the pre-specified criteria (data not shown). Therefore, we conclude that single dose EP2006 and US-Neupogen PK are similar. The PK time vs concentration profile is depicted in Figure 4.

Table 4. Summary of single dose PK parameter values (study EP06-109).

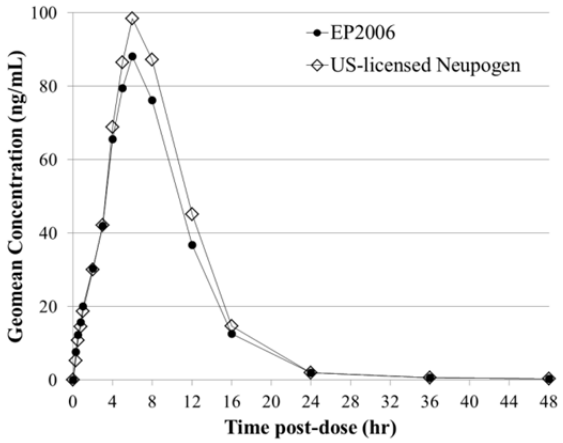
Treatment (N)	Geomean (Geo CV%) $AUC_{0\text{-last}}$ (ng*h/mL)	Geomean (Geo CV%) C_{max} (ng/mL)	Median (range) T_{max} (h)	Geomean (Geo CV%) t½ (h)
EP2006 (N=26)	924 (20)	90 (23)	6 (4, 8)	8.6 (11.7)
US-Neupogen (N=26)	1044 (20)	100 (24)	6 (4, 8)	8.3 (13.7)

Table 5. Statistical analyses for assessment of PK similarity (study EP06-109).

Parameter	Geometric Mean Ratio (90% CI)
$AUC_{0\text{-last}}$	88 (84, 91)
C_{max}	88 (84, 92)

Ratio (%): EP2006/US-Neupogen

Figure 4. Geometric mean G-CSF time vs. concentration profile (study EP06-109).



Differences around the T_{max} between EP2006 and US-Neupogen (Figure 4) appear to be related to differences in the buffer systems between the products, where EP2006 uses a glutamate buffer and US-Neupogen an acetate buffer. Study EP06-104 confirmed this hypothesis. Study EP06-104 was a 3-way cross-over study that evaluated EU-Neupogen (acetate) and two EP2006 (glutamate and acetate) formulations. Single 2.5 mcg/kg SC doses were administered to healthy subjects (N=28). The time vs concentration time profile is depicted in Figure 5 shows that EP2006 acetate has a superimposable profile with EU-approved Neupogen. Also, the statistical analyses confirm the similarity in AUC and C_{max} between the acetate formulations (Table 6). Differences in PK between the acetate and glutamate buffer formulations did not translate into differences in PD (ANC) (Table 6).

Figure 5. Mean G-CSF time vs. concentration profile (study EP06-104).

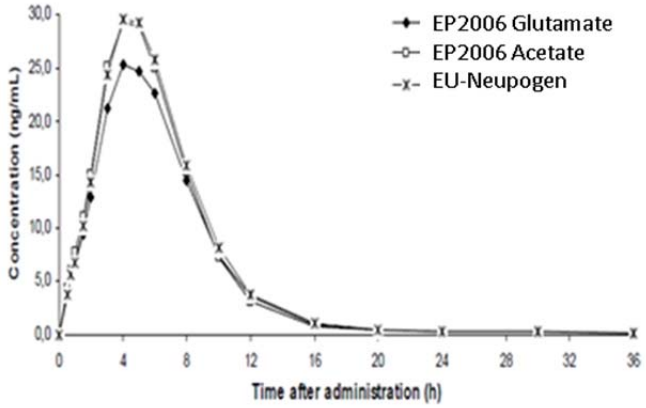


Table 6. Statistical Analyses of PK and PD (ANC) parameters between treatments (study EP06-104)

Treatment A	Treatment B	PK Geometric Mean Ratio (90% CI)		PD (ANC) Geometric Mean Ratio (95% CI)	
		$AUC_{0\text{-last}}$	C_{max}	$AUEC_{0\text{-last}}$	ANC_{max}
EP2006 Acetate	EU-Neupogen	99 (92, 106)	102 (94, 111)	101 (96, 105)	100 (95, 106)
EP2006 Glutamate	EP2006 Acetate	91 (85, 98)	83 (76, 90)	101 (96, 105)	96 (91, 102)
EP2006 Glutamate	EU-Neupogen	89 (83, 96)	84 (78, 92)	101 (97, 106)	97 (91, 103)

Ratio (%): Treatment A/Treatment B

US-licensed Neupogen single PD (ANC) results

Single dose PD (ANC) was assessed in study EP06-109 where single 10 mcg/kg SC doses of EP2006 and US-Neupogen were administered to healthy subjects. Table 7 summarizes the ANC parameter values. The ANC AUEC and ANC C_{max} geometric mean ratios and 95% CI met the pre-specified 80-125% criteria (Table 8). Of note, the 90% CI, 80-125% similarity limits for ANC were also met (results not shown). Therefore, we conclude that single dose EP2006 and US-Neupogen PD (ANC) are similar. The ANC time vs concentration profile is depicted in Figure 6.

Table 7. Summary of single dose ANC parameter values (study EP06-109).

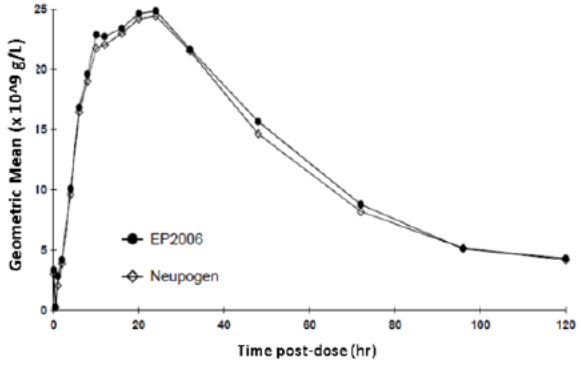
Treatment (N)	Geomean (Geo CV%) $AUEC_{0\text{-}120h}$ ($h*10^9/L$)	Geomean (Geo CV%) C_{max} ($10^9/L$)	Median (range) T_{max} (h)
EP2006 (N=26)	1524 (16)	26 (18)	20 (10, 24)
US-Neupogen (N=26)	1472 (18)	25 (20)	20 (12, 24)

Table 8. Statistical analyses for assessment of ANC similarity (study EP06-109).

Parameter	Geometric Mean Ratio (95% CI)
ANC $AUEC_{0\text{-last}}$	103 (100, 106)
ANC_{max}	100 (96, 103)

Ratio (%): EP2006/US-Neupogen

Figure 6. Geometric mean ANC time vs. concentration profile (study EP06-109).



EU-approved single dose PK and PD (ANC) results

EP2006 also showed PK and ANC similarity when compared to the EU-approved Neupogen following single SC doses of 1, 2.5, 5 and 10 mcg/kg in healthy subjects. The predefined PK similarity limits were met for both AUC and C_{max} (90% CI, 80-125%) at all doses, except for C_{max} at the 2.5 mcg/kg dose where the PK similarity was missed marginally (Table 9). The predefined PD similarity limits for AUEC and ANC_{max} were met for ANC (95% CI, 80-125%) (Table 9). Of note, the 90% CI, 80-125% similarity limits for ANC were also met (results not shown). Therefore, we conclude that single dose EP2006 and EU-Neupogen PK and PD (ANC) are similar. The results from these EU-Neupogen studies are consistent with the results from study EP06-109, described above, where US-licensed Neupogen was used. These studies are also supportive of the the scientific bridge to US-licensed Neupogen; refer to CMC review for details regarding the scientific bridge.

Table 9. Summary of single dose PK and ANC similarity statistical analysis results from studies using EU-approved Neupogen.

Study (N)	Dose (mcg/kg)	PK Geometric Mean Ratio (90% CI)	ANC Geometric Mean Ratio (95% CI)
EP06-105 (N=23)	1	AUC_{0-36h}: 91 (86, 97) C_{max}: 89 (82, 96)	$AUEC_{0-120h}$: 102 (97, 109) ANC_{max}: 100 (94, 105)
EP06-103	2.5 (N=28)	AUC_{0-24h}: 88 (81, 85) C_{max}: 87 (79*, 95)	$AUEC_{0-24h}$: 102 (99, 105) ANC_{max}: 104 (97, 111)
	5 (N=27)	AUC_{0-24h}: 96 (90, 102) C_{max}: 96 (89, 104)	$AUEC_{0-24h}$: 101 (98, 103) ANC_{max}: 100 (95, 105)
EP06-101 (N=32)	10	AUC_{0-24h}: 93 (89, 98) C_{max}: 89 (82, 96)	Single dose ANC not reported

*The lower limit for Cmax at the 2.5 mcg/kg dose fell just outside the 80-125% range.
Ratio (%): EP2006/EU-Neupogen

Multiple dose PD ($CD34^+$ cell counts)

EU-approved Neupogen multiple dose PD ($CD34^+$) results

Multiple dose PD ($CD34^+$) was assessed in studies EP06-101 and EP06-103 where multiple (7 daily) 2.5, 5, and 10 mcg/kg SC doses of EP2006 and EU-Neupogen were administered to healthy subjects. Table 10 summarizes the $CD34^+$ parameter values. The PD similarity limits for $AUEC_{0-216h}$ and $CD34_{max}$ were met for $CD34^+$ (95% CI, 80-125%) (Table 11). Of note, the 90% CI, 80-125% similarity limits for $CD34^+$ were also met (results not shown). Therefore, we conclude that single dose EP2006 and EU-Neupogen PK and PD (ANC) are similar. The $CD34^+$ time vs concentration profile for the 10 mcg/kg dose in study EP06302 is depicted in Figure 7.

Table 10. Summary of multiple dose $CD34^+$ parameter values (studies EP06-101 and -103).

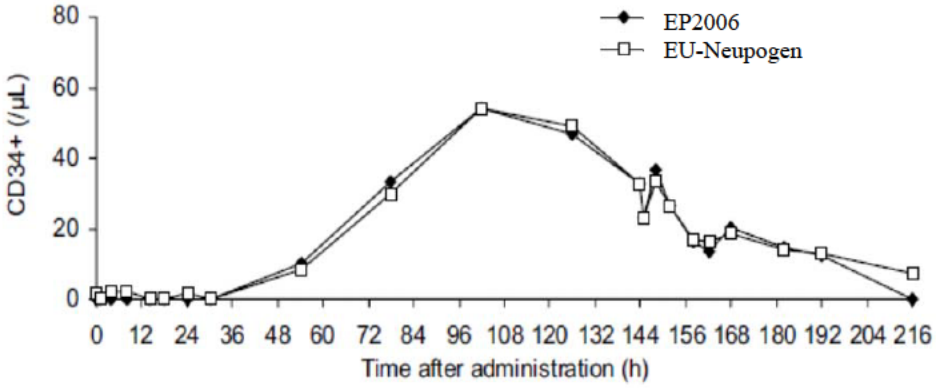
Study (N)	SC Dose (mcg/kg)	Geomean (CV%) $AUEC_{0\text{-}216h}$		Geomean (CV%) $CD34_{max}$	
		EP2006	EU-Neupogen	EP2006	EU-Neupogen
EP06-103	2.5 (N=28)	2815 (82)	2694 (92)	33 (73)	31 (81)
	5 (N=27)	2886 (79)	2898 (77)	37 (77)	37 (75)
EP06-101 (N=32)	10	5129 (52)	5023 (57)	58 (47)	58 (50)

Table 11. Summary of multiple dose $CD34^+$ similarity statistical analysis results from studies using EU-approved Neupogen (studies EP06-101 and -103).

Study	SC Dose (mcg/kg)	Geometric Mean Ratio (95% CI)	
		$AUEC_{0\text{-}216h}$	$CD34_{max}$
EP06-103	2.5	105 (97, 113)	99 (84, 117)
	5	99 (87, 113)	99 (84, 117)
EP06-101	10	102 (95, 110)	99 (90, 110)

Ratio (%): EP2006/EU-Neupogen

Figure 7. Multiple dose geometric mean $CD34^+$ time vs. concentration profile (study EP06-101).



2.2.6.2 How does the PK and PD (ANC) of the drug in healthy volunteers compare to that in patients?

Patient PK substudy:

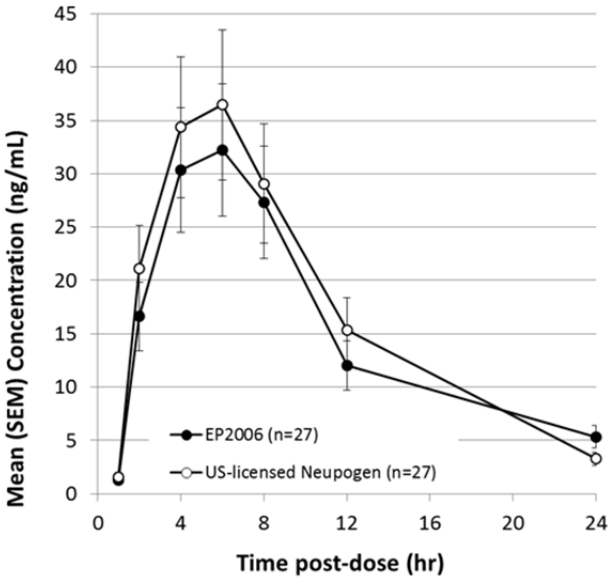
US-licensed Neupogen single dose PK and PD (ANC) results

As part of the comparative clinical study EP06-302, the applicant included an exploratory PK sub-study (n=54; 27 per arm) in order to describe the PK of EP2006 and the reference product (US-licensed Neupogen) following a single 5 mcg/kg SC dose in Cycle 1 in patients. EP06-302 was a parallel design study intended to evaluate the efficacy and safety of EP2006 vs. US-licensed Neupogen in patients. The study was not intended to evaluate the PK similarity of EP2006 to US-licensed Neupogen. EP2006 or US-licensed Neupogen was administered daily, starting on Day 2 of each chemotherapy cycle (at least 24 hours after chemotherapy ended) and continued until the ANC recovered to 10×10^9/L

after the nadir or up to a maximum of 14 days (whichever occurred first); chemotherapy cycles were three weeks apart.

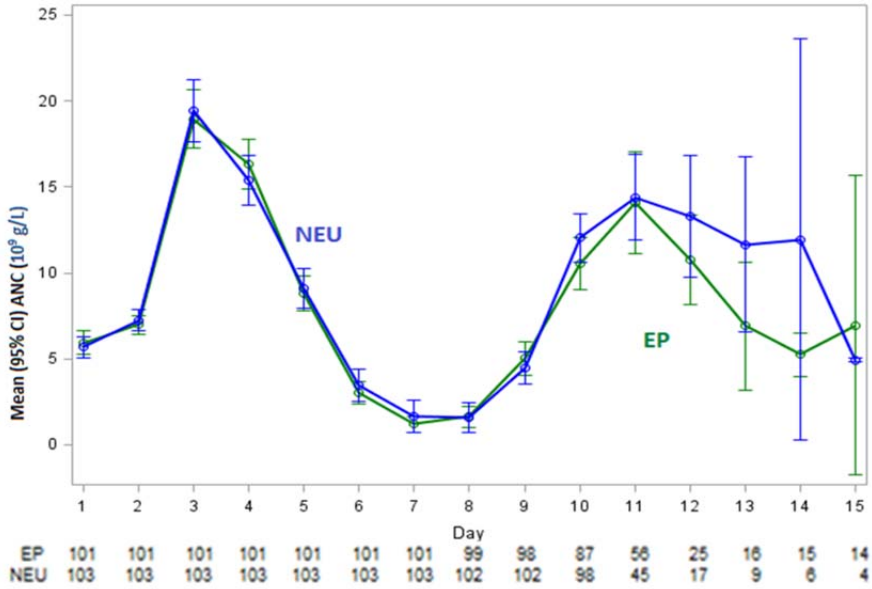
In the exploratory substudy, the exposure (AUC and C_{max}) of EP2006 was lower than that observed for US-licensed Neupogen (Figure 8). The inter-subject coefficient of variability (CV%) observed in this sub-study was around 40%, which was greater than that observed in the healthy subject studies (around 20%). The PK sub-study was a parallel design study whereas the dedicated PK similarity assessment was a crossover design study. The PK sub-study arms were well balanced and comparable to that of the per protocol study arms as it relates to baseline demographics, baseline clinical laboratory values, actual doses administered, and chemotherapy received. Analyzing the stratum of adjuvant versus neoadjuvant chemotherapy did not account for the differences observed in PK either.

Figure 8. Cycle 1 mean time vs. concentration time-profile in patients (study EP06-302).



The differences observed in PK in patients in Cycle 1 did not appear to translate into clinically meaningful PD differences. The time course of the ANC in Cycle 1 is illustrated in Figure 9. The nadir occurred on Days 7 and 8, which is as expected. There were no marked differences in the mean ANC profile between EP2006 and US-licensed Neupogen up to Day 10. However, following Day 10, when the ANC had recovered by reaching at least 10 x 10^9/L in most patients, the number of patients with PD measurements decreased markedly. Of note, per protocol, ANC measurements were only made until the ANC recovered or until Day 15, whichever occurred first. Therefore, the difference in ANC profiles beyond day 10 is likely influenced by low patient numbers. The depth and the time of the ANC nadir in Cycle 1 were also similar in patients receiving EP2006 and patients receiving US-licensed Neupogen (refer to clinical review for results). Also, refer to the clinical review for a description of the overall efficacy and safety results from Study EP06-302.

Figure 9. Daily mean (95% CI) ANC in Cycle 1 (study EP06-302).



The number of subjects in each arm and at each time point is shown at the bottom of the graph.

2.3 INTRINSIC FACTORS

2.3.3 Immunogenicity

2.3.3.1 What is the incidence (rate) of the formation of the anti-product antibodies (APA), including the rate of pre-existing antibodies, the rate of APA formation during and after the treatment, time profiles and adequacy of the sampling schedule?

Patients were tested for anti-product antibodies (APAs) in all clinical trials. All serum samples were screened using a radioimmunoprecipitation (RIP) assay, without spiking of unlabelled drug. Samples with binding values above the screening cut-point were then reanalyzed in a confirmatory RIP assay using unlabeled rhG-CSF (EP2006 or Neupogen® as unlabelled protein). Specificity of the binding was confirmed if the rate of depletion of the bound radioactivity was above the above the validated specificity cut-point when unlabeled rhG-CSF was added to the medium. Samples positive for binding antibodies in the confirmatory RIP assay were evaluated for neutralizing anti-rhG-CSF antibodies in a cell-based neutralization antibody assay (NAB).

Immunogenicity sampling schedule in the studies was adequate. The following is the sampling time points for the PK similarity studies:

- Screening (or pre-dose Period 1)
- Pre-dose Period 2
- Follow-up Visit

The following is the sampling time points for the comparative clinical study EP06-302:

- Pre-dose (chemotherapy) in each Cycle (Cycles 1-6)

- End of treatment (day 21 of Cycle 6)
- Study termination visit (4wk after the last dose)

No samples were confirmed positive after being tested using the confirmatory assay. The overall APA incidence to G-CSF (i.e., any post-dose time) within each study was <1%.

The presence of mAb in patient serum at the time of ATA sampling can interfere with the ability of this assay to detect ATA. As a result, data may not accurately reflect the true incidence of ATA development.

Refer to the CMC immunogenicity review for more details.

2.3.3.2 Does the immunogenicity affect the PK and/or PD of the therapeutic protein?
Conclusions regarding the impact of immunogenicity on G-CSF PK or PD cannot be drawn at this time due to the low immunogenicity incidence rate (see section 2.3.3.1).

2.3.3.3 Do the anti-product antibodies have neutralizing activity?
No samples were confirmed to be APA positive so neutralizing activity of APA was not assessed (see section 2.3.3.1).

2.3.3.4 What is the impact of anti-product antibodies on clinical efficacy?
The impact of APA on clinical efficacy is limited due to the low incidence rate of APA following G-CSF treatment (see section 2.3.3.1).

2.3.3.5 What is the impact of anti-product antibodies on clinical safety? *(e.g., infusion-related reactions, hypersensitivity reactions, cross-reactivity to endogenous counterparts, etc.)?*
The impact of APA on clinical safety is limited due to the low incidence rate of ATA following G-CSF treatment (see section 2.3.3.1).

2.6 ANALYTICAL SECTION

2.6.1 How are the active moieties identified and measured in the clinical pharmacology and biopharmaceutics studies?
Serum G-CSF concentrations were measured in plasma by a validated Enzyme-Linked Immunosorbent Assay (ELISA). The enzyme immunoassay kit is a sandwich assay with a monoclonal capture antibody and a polyclonal detection antibody. In the first step, G-CSF is captured by anti-G-CSF antibodies (mouse, monoclonal) bound to the wells of a microtiter plate. In the second step, horseradish-peroxidase-labeled anti-G-CSF antibodies (goat, polyclonal) are bound to G-CSF. After incubation with the substrate, tetramethylbenzidine (TMB), the reaction is stopped by the addition of sulphuric acid. The absorption is read photometrically. Validation reports were submitted and QC reports were summarized for the use of the method for each study.

<u>PD assays:</u>

- ANC was measured with hematology analyzers or flow cytometry. All assays were validated and reports were submitted.

- CD34+ was measured with flow cytometry. All assays were validated and reports were submitted.

2.6.2 Which metabolites have been selected for analysis and why?
Not applicable

2.6.3 For all moieties measured, is free, bound, or total measured? What is the basis for that decision, if any, and is it appropriate?
Not applicable.

2.6.4 What bioanalytical methods are used to assess therapeutic protein concentrations? Briefly describe the methods and summarize the assay performance.
Serum G-CSF concentrations were measured in plasma by a validated ELISA. See section 2.6.1 above.

The accuracy, precision, and other relevant parameters for the assay are described in Table 12. This is sufficient to meet the requirements of the submitted studies.

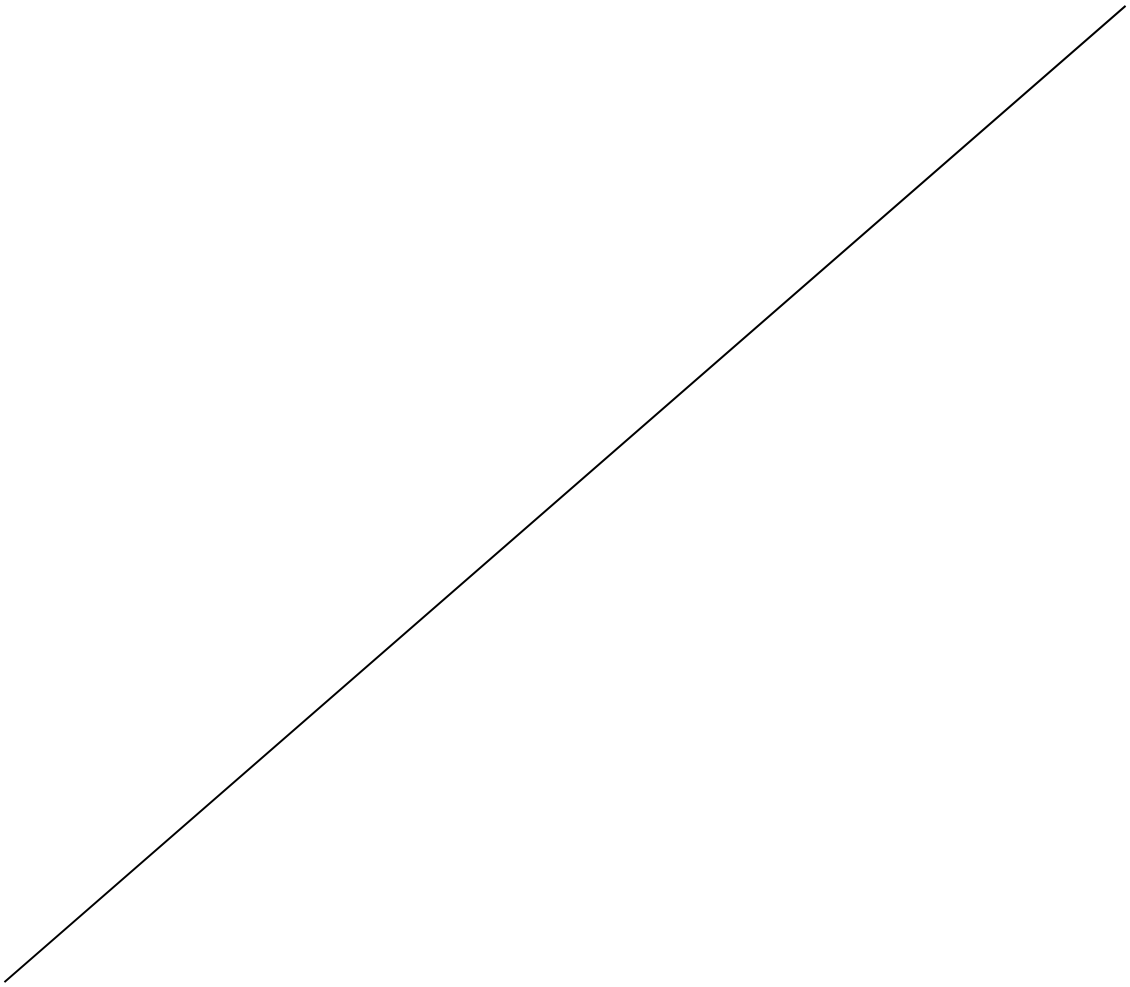


Table 12. Summary of the G-CSF ELISA validation results.

Parameter	Acceptance criteria	Results	Compliance
Linearity of calibration curve (analytical range, 7 levels, N=5, duplicates)	Accuracy: Cal 1 (LLOQ): ± 20% Cal 2 to Cal 7: ± 15% Precision: Cal 1 (LLOQ): CV ≤ 20% Cal 2 to Cal 7: CV ≤ 15%	Acceptance criteria always fulfilled. Analytical range from 39 pg/mL to 2500 pg/mL confirmed.	Passed
Sensitivity/Lower limit of quantification (LLOQ, 1 level, N=5, duplicates)	Accuracy: ± 20% Precision: CV ≤ 20%	39 pg/mL (Cal 1): Accuracy: 2.3% Precision: 8.3% → 0.039 ng/mL could be analyzed with acceptable accuracy and precision.	 Passed Passed
Intra-assay precision (3 or 4 levels, N=3 or 5)	QC samples from the kit manufacturer: QC1, QC2: CV ≤ 15% QC3: CV ≤ 20% Incurred samples: BQC1, BQC2, BQC3 CV ≤ 15% BCQ4: CV ≤ 20% Spiked QC samples: SQC1, SQC2: ≤ 15%	 QC1, QC2: 3.2% to 8.5% QC3: 5.5% BQC1, BQC2, BQC3: 3.6% to 9.9% BQC4: 9.9% SQC1, SQC2: 2.2% to 4.5%	 Passed Passed Passed Passed Passed
Inter-assay precision (3 or 4 levels, N=3 or 5, 5 days)	QC samples from the kit manufacturer: QC1, QC2: CV ≤ 15% QC3: CV ≤ 20% Incurred samples: BQC1, BQC2, BQC3 CV ≤ 15% BCQ4: CV ≤ 20% Spiked QC samples: SQC1, SQC2: ± 15%	 QC1, QC2: 3.9% to 5.4% QC3: 5.5% BQC1, BQC2, BQC3: 7.1% to 8.7% BQC4: 14.5% SQC1, SQC2: 6.7% to 7.5%	 Passed Passed Passed Passed Passed
Accuracy (2 levels, N=5)	Spiked QC samples: SQC1, SQC2: ± 15%	Inter-assay: 2.2% and 5.4% Intra-assay: -1.8% and -1.6%	Passed Passed
Dilution linearity (Dilution factor: 10, 50, 100, 200, N=5)	Mean accuracy: ± 15%	Mean accuracy: 1: 10: 3.6% 1: 50: 6.5% 1: 100: 0.2% 1: 200: 2.7%	 Passed Passed Passed Passed

Parameter	Acceptance criteria	Results	Compliance
	Precision: CV ≤ 15%	Precision:	
		1: 10: CV: 7.5%	Passed
		1: 50: CV: 3.9%	Passed
		1: 100: CV: 5.8%	Passed
		1: 200: CV: 7.4%	Passed
Stability	Mean accuracy: ± 15%	Mean accuracy:	
- Short-term (2 h/4 h),		- Short-term: -5.1% to 12.2%	Passed
		- Freeze-thaw: -3.7% to 2.3%	Passed
- Freeze-thaw (once, twice, thrice);		- Long-term -20°C: -8.1% to 4.9%	Passed
		- Long-term -70°C: -7.3% to 4.2%	Passed
- Long-term (-20°C/-70°C; 2 days, 2 months, 14 months)	Precision: CV ≤ 15%	Precision (CV):	
		- Short-term: 2.7% to 10.0%	Passed
		- Freeze-thaw: 2.8% to 11.4%	Passed
		- Long-term -20°C: 2.6% to 9.3%	Passed
		- Long-term -70°C: 1.6% to 10.0%	Passed
(BQC1, BQC3 and AQC1 used)	95% ANOVA confidence interval of the accuracy results: stability concluded if both the upper and lower limit of the CI ≤ 15%		Passed for all stability tests
Hemolyzed human serum (N=5)	Mean accuracy: ± 15%	Mean accuracy: 6.3%	Passed
	Precision: CV ≤ 15%	Precision: CV: 5.4%	Passed
Drug-free human samples (24 samples tested)	N/A	17/24 samples (71%): < LLOQ 7/24 samples (29%): endogenous G-CSF concentration range: 0.0423 to 0.0736 ng/mL.	N/A
Incurred human serum samples (45 samples from Study EP06-102 tested)	Mean percentual deviation (%) of absolute percentual difference (%) of the 1st and 2nd analysis: ≤ 20%	Mean deviation: 6.7%	Passed
Recovery of EP2006 in comparison to Neupogen® (EU-approved)	Precision: CV ≤ 15%	Precision: 3.3% to 9.0%	Passed
	Mean EP2006/ Neupogen® ratio: 0.95 to 1.05	Mean EP2006/ Neupogen® ratio:	
(4 levels, 0.05-1 ng/mL, N=5, 3 days)		0.98 to 1.00	Passed

Cal (Calibrator): Cal 1 (0.0390 ng/mL), Cal 2 (0.0780 ng/mL), Cal 3 (0.156 ng/mL), Cal 4 (0.312 ng/mL), Cal 5 (0.625 ng/mL), Cal 6 (1.25 ng/mL), Cal 7 (2.50 ng/mL)

QC (QC samples from the kit manufacturer (Quality controls)): QC1 (1.43-1.95 ng/mL), QC2 (0.726-1.00 ng/mL), QC3 (0.244-0.376 ng/mL)

2.6.5 What is the range of the standard curve? How does it relate to the requirements for clinical studies? What curve fitting techniques are used?

The ELISA methods developed are discussed in Section 2.6.1. The range of the standard curve is 0.03900 to 2.500 ng/mL and is also described in Table 12. Using the theoretical concentrations of the standards and measured absorbance a four parameter marquardt

regression was performed. The assay range combined with the validated dilution methods are acceptable based on serum G-CSF concentrations observed in the studies.

2.6.5 What is the QC sample plan?

Quality Control (QC) samples were freshly prepared on each analysis day by spiking the respective working solutions into human serum. Three concentrations in the range of the standard curve were used. The run was accepted, if the accuracy of 2/3 of the control and spiked control samples (QC 1 and QC 2; Spiked QC Standard (SQCWS) 1 and SQCWS 2) was within the acceptance range of the control samples and within 85 to 115 % of the theoretical concentration for the spiked quality control samples. Refer to Table 12 for a summary of the between-run accuracy and precision of QC samples for G-CSF.

2.6.6 What bioanalytical methods are used to assess the formation of the anti-product antibodies? Briefly describe the methods and assay performance including sensitivity, specificity, precision, cut point, interference and matrix, etc.

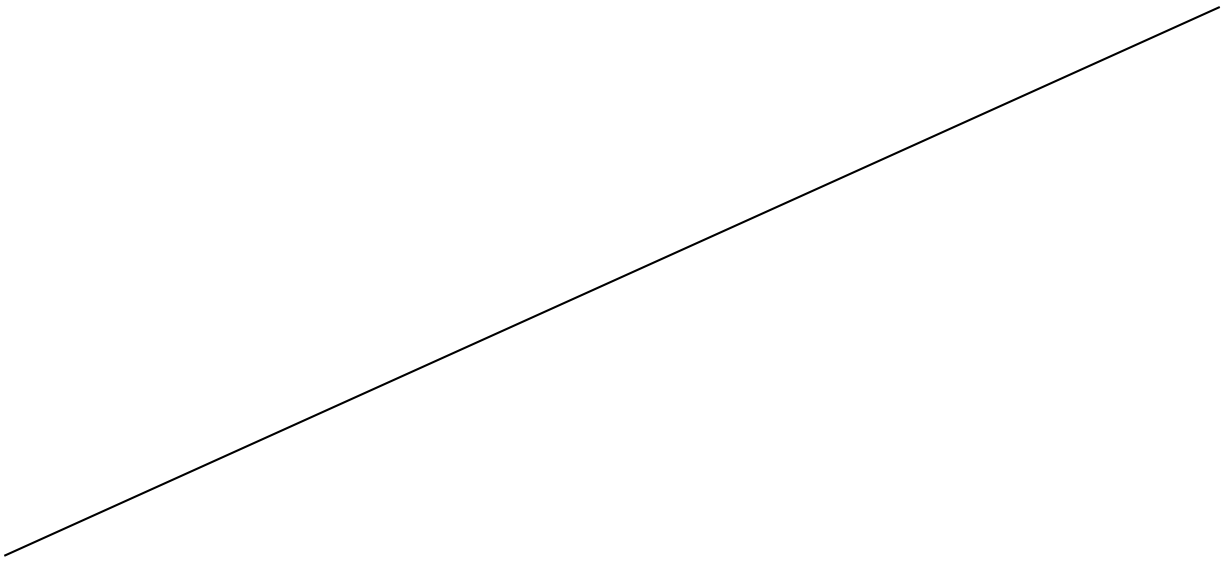
The clinical trials used a radioimmunoprecipitation (RIP) assay, without spiking of unlabelled drug, to test for anti-rh-G-CSF antibdoies. Samples with binding values above the screening cut-point were then reanalyzed in a confirmatory RIP assay using unlabeled rhG-CSF (EP2006 or Neupogen® as unlabelled protein). Specificity of the binding was confirmed if the rate of depletion of the bound radioactivity was above the above the validated specificity cut-point when unlabeled rhG-CSF was added to the medium. Refer to the CMC immunogenicity review for further details on the assays.

2.6.6.1 What is the performance of the binding assay(s)?

Refer to the CMC immunogenicity review for information and details regarding the performance of the binding assays.

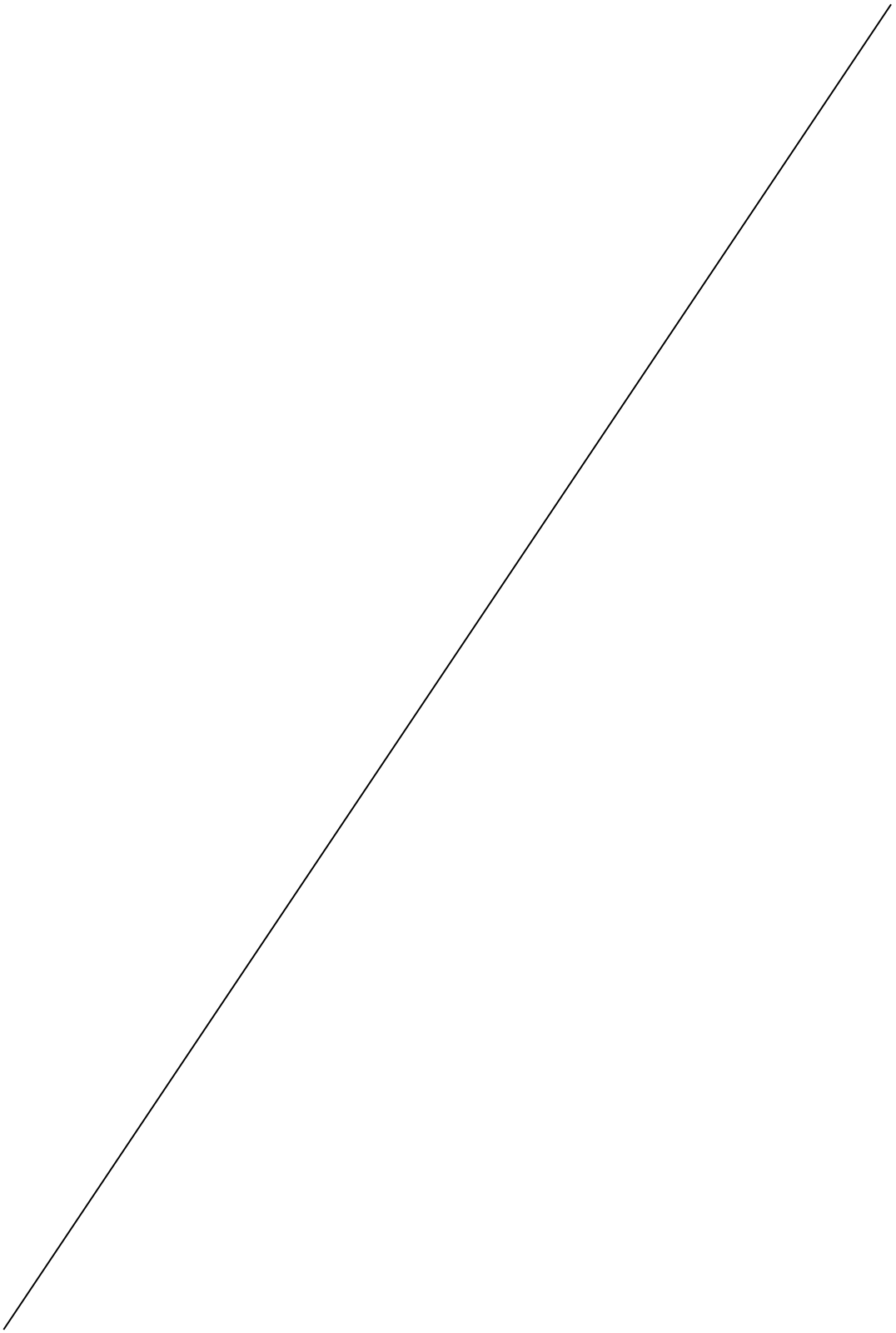
2.6.6.2 What is the performance of the neutralizing assay(s)?

Samples positive for binding antibodies in the confirmatory RIP assay were evaluated for neutralizing anti-rhG-CSF antibodies in a cell-based neutralization antibody assay (NAB). However, given that samples did not test positive for anti-rh-G-CSF antibodies, the neutralizing assay was not used.



DETAILED LABELING RECOMMENDATIONS

- Refer to the approved US-licensed Neupogen label



3 APPENDIX

3.1 FDA Briefing Document Oncologic Drugs Advisory Committee Meeting January 7, 2015 for BLA 125553: Clinical Pharmacology Section.

Refer to the Advisory Committee Briefing Materials Located at: http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/OncologicDrugsAdvisoryCommittee/UCM428780.pdf

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/s/

SARAH J SCHRIEBER
01/27/2015

ANSHU MARATHE
01/29/2015

VIKRAM P SINHA
01/29/2015

NAM ATIQUR RAHMAN
01/29/2015
I accept the recommendation of the review team.